

## CLAIMS

I claim:

1. A method of producing refolded recombinant pro-urokinase, comprising:
  - (a) solubilizing pro-urokinase protein with a solubilization buffer, said solubilization buffer comprising a high concentration of chaotroph, a reducing agent, and having a pH of about 9.0 to about 11.0, thereby producing a solubilized pro-urokinase solution;
  - (b) rapidly diluting said solubilized pro-urokinase solution with refolding buffer by adding said solubilized pro-urokinase solution into the refolding buffer, thereby producing a diluted solubilized pro-urokinase solution;
  - (c) reducing the pH of the diluted solubilized pro-urokinase solution to a pH of about 7.5 to about 8.5, wherein said pH reducing is carried out over a period of at least about 20 hours, thereby producing refolded pro-urokinase.
2. The method of claim 1, wherein said pro-urokinase is human pro-urokinase.
3. The method of claim 1, wherein said chaotroph is urea.
4. The method of claim 3, wherein said urea is at about 8 M concentration.
5. The method of claim 1, wherein said chaotroph is guanidine hydrochloride..
6. The method of claim 5, wherein said guanidine hydrochloride is at about 6 M concentration.
7. The method of claim 1, wherein said solubilization buffer is pH about 10.0 to about 10.5.
8. The method of claim 1, wherein the pH of the diluted solubilized pro-urokinase solution is reduced to about pH 8.0.

9. The method of claim 1, said method further comprising adjusting the A<sub>280</sub> of the solubilized pro-urokinase solution to about 2.0 to about 5.0 before step (b).

10. The method of claim 9, wherein the A<sub>280</sub> of the solubilized pro-urokinase solution is adjusted by diluting the solubilized pro-urokinase solution in a buffer comprising about 8 M urea, about 10 mM β-mercaptoethanol, about 10 mM dithiothreitol (DTT), about 1 mM reduced glutathion (GSH), and about 0.1 mM oxidized glutathion (GSSG) at pH about 10 to about 10.5.

11. The method of claim 1, wherein the solubilized pro-urokinase solution is diluted into about twenty-fold refolding buffer.

12. The method of claim 1, wherein the refolding buffer comprises urea at about 0.8 M to about 2.5 M and arginine at about 0.05 M to about 1.5 M.

13. The method of claim 12, wherein said refolding buffer comprises urea at about 2.0 M and arginine at about 0.2 M.

14. The method of claim 1, wherein the refolding buffer comprises guanidine HCl at about 1 M and arginine at about 0.2 M.

15. The method of claim 1, wherein said solubilization buffer comprises about 8 M urea and about 100 mM beta-mercaptoethanol and has a pH of about 10.5; said solubilized pro-urokinase solution is adjusted to A<sub>280</sub> of about 5.0 then rapidly diluted into about 20 fold refolding buffer, and said refolding buffer comprises about 2 M urea, about 0.2 M arginine.

16. The method of claim 1, wherein said solubilization buffer comprises about 8 M urea and about 100 mM beta-mercaptoethanol and has a pH of about 10.5; said solubilized pro-urokinase solution is adjusted to A<sub>280</sub> of about 5.0 then rapidly diluted into about 20 fold refolding buffer, and said refolding buffer comprises about 1 M guanidine HCl, about 0.2 M arginine.

17. The method of claim 1, further comprising lysing bacterial host cells comprising denatured pro-urokinase protein and collecting said denatured pro-urokinase protein.
18. The method of claim 17, further comprising washing said denatured pro-urokinase protein.
19. The method of claim 1, further comprising purifying said refolded pro-urokinase.
20. The method of claim 19, wherein said refolded pro-urokinase is purified by size exclusion chromatography (SEC).
21. The method of claim 19, wherein said refolded pro-urokinase is purified by ion exchange chromatography (IEC).
22. The method of claim 19, wherein said refolded pro-urokinase is purified by heparin affinity chromatography.
23. The method of claim 19, wherein said refolded pro-urokinase is purified by hydroxyapatite chromatography.
24. A composition comprising refolded pro-urokinase, produced by the method of claim 1.